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## A biomimetic receptor for glucose

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**Specific molecular recognition is routine for biology, but has proved difficult to achieve in synthetic systems. Carbohydrate substrates are especially challenging, because of their diversity and similarity to water, the biological solvent. Here we report a synthetic receptor for glucose, which is biomimetic in both design and capabilities. The core structure is simple and symmetrical, yet provides a cavity which almost perfectly complements the all-equatorial  $\beta$ -pyranoside substrate. The affinity for glucose, at  $K_a \sim 18,000 \text{ M}^{-1}$ , compares well with natural receptor systems. Selectivities also reach biological levels. Most other saccharides are bound  $\sim 100$  times more weakly, while non-carbohydrate substrates are ignored. Glucose-binding molecules are required for initiatives in diabetes treatment, such as continuous glucose monitoring and glucose-responsive insulin. The performance and tunability of this system augur well for such applications.**

The selective recognition of complex molecules is a hallmark of biology<sup>1</sup>. Evolution can create binding sites with precise complementarity to substrates, capable of strong and specific complexation. In principle, designed host molecules should be capable of similar behaviour, but in practice this has been difficult to realise. Synthetic receptors have been widely studied as models of biomolecular recognition<sup>2,3</sup>, but have rarely achieved performance levels which compete with biomolecules and allow substrate targeting in biological media<sup>4-7</sup>. The problems are especially acute for the binding of polar molecules, which are strongly hydrated and must compete with water for polar binding groups<sup>6-8</sup>. Here we describe a synthetic receptor which binds glucose, a medically important substrate<sup>9,10</sup>, with performance levels that match most biological counterparts. The results show that designed abiotic hosts can achieve both qualitative and quantitative biomimicry, even when facing the most difficult tasks in molecular recognition.

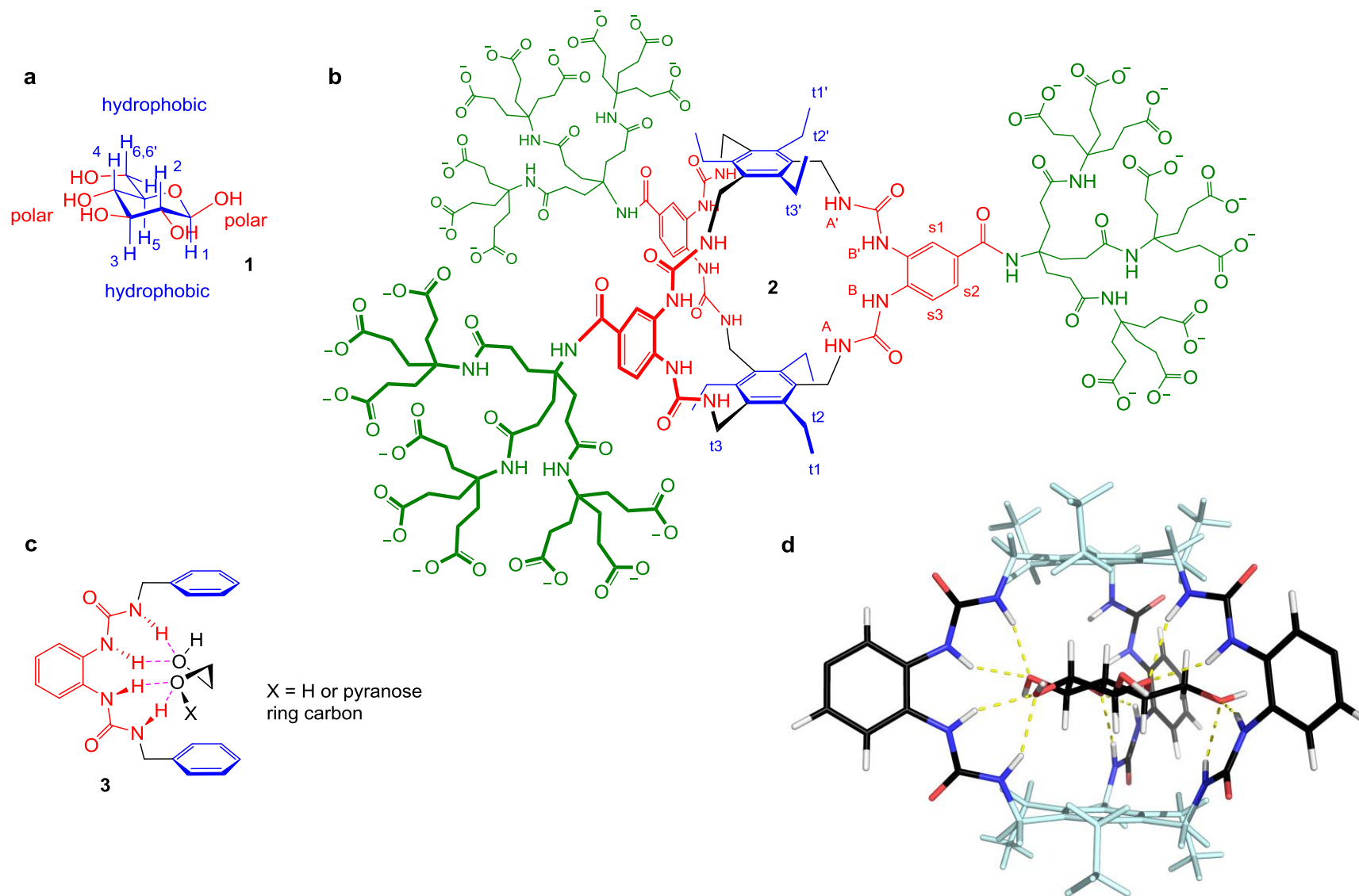
The binding of carbohydrates in water is an especially challenging problem, both for chemists and for natural systems<sup>11,12</sup>. Saccharides are both hydrophilic and hydromimetic (resembling water) so are difficult to distinguish from surrounding solvent. They also possess complex three-dimensional structures which must be differentiated to achieve useful selectivity. Carbohydrate-binding proteins (e.g. lectins) tend to show low affinities, and often quite modest selectivities<sup>13</sup>. For example the lectin commonly employed for glucose, Concanavalin A (Con A), binds with  $K_a \sim 500 \text{ M}^{-1}$  and also targets mannose<sup>14</sup>. Synthetic lectin mimics have been designed by

ourselves<sup>8,15-19</sup> and others<sup>11,12</sup>, but are generally still weaker. The record for glucose currently stands at  $K_a \sim 250 \text{ M}^{-1}$ ,<sup>19</sup> while glucose/galactose selectivity is typically  $\sim 10$ . Moreover selectivity against non-carbohydrates may be poor. Binding sites complementary to saccharides can also match other small molecules, sometimes leading to much higher affinities<sup>20</sup>. Such off-target binding could be especially damaging for real-world applications in complex biological media. Receptors which incorporate boronic acids may bind more strongly, but tend to complex polyols in general and to show pH-sensitivity<sup>9,21</sup>.

A carbohydrate molecule presents an array of specifically positioned polar groups (mostly hydroxyl) with small hydrophobic regions composed of CH groups. Following the lead of biology<sup>12</sup>, a carbohydrate binding site should complement the hydroxyl groups with hydrogen bonding units, and the hydrophobic regions with aromatic surfaces capable of CH- $\pi$  interactions. In the case of glucose, the predominant  $\beta$ -pyranose form **1** possesses an all-equatorial arrangement of polar substituents and two hydrophobic patches composed of axial CH groups (Fig. 1a). In previous work, we have developed a general approach to binding all-equatorial carbohydrates, involving cavities composed of parallel aromatic surfaces separated by spacers containing amide linkages<sup>15-19</sup>. However, while the spacers can provide occasional hydrogen bonds, they have not been specifically positioned to promote binding and selectivity.

Here we present a lectin mimic in which, for the first time, hydrogen bonding has been extensively and rationally integrated into the design. Bicyclic cage **2** (Fig. 1b) features

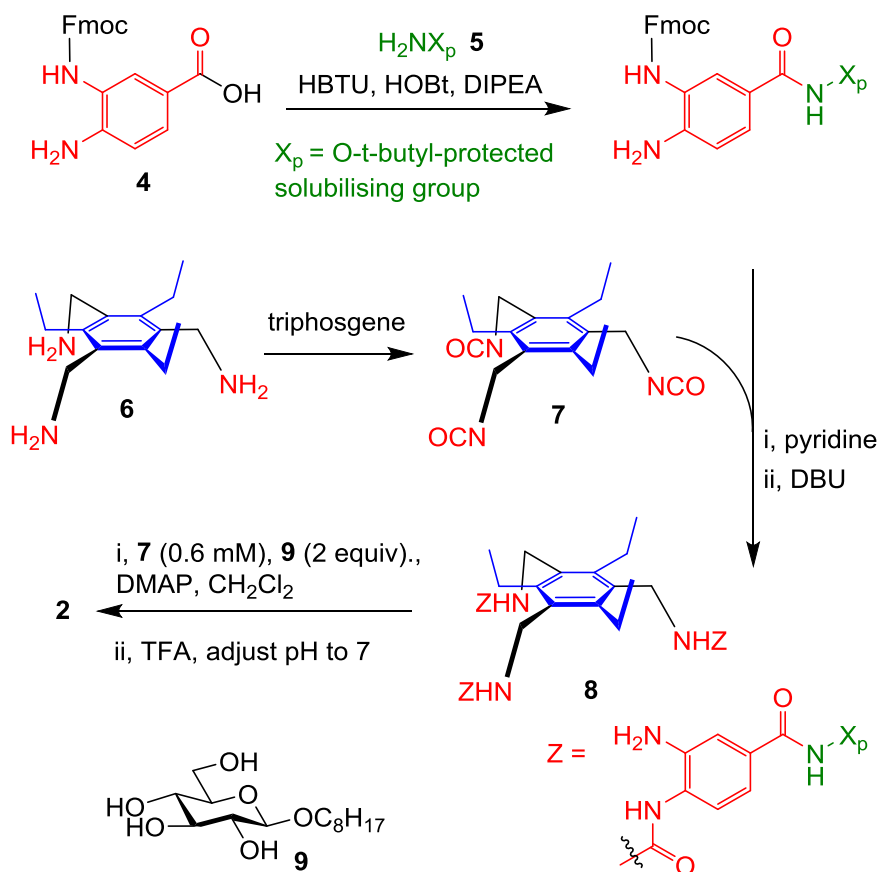
six urea groups, providing an exceptionally dense array of polar functionality. Triethylmesitylene (TEM) units<sup>22-24</sup> serve as roof and floor, positioned to form hydrophobic/CH- $\pi$  interactions with  $\beta$ -glucose CH. Three peripheral nonacarboxylates are added to maintain water-solubility. Modelling of the empty receptor (see Supplementary Information) yields structures in which all urea NH groups point inwards, despite the potential for intramolecular hydrogen bonding within spacers **3** (Fig. 1c). The spacers hold the TEM rings  $\sim 8.4$  Å apart. Previous work has shown that this separation is close to ideal for accommodating an all-equatorial carbohydrate<sup>18</sup>. It is also significantly larger than required for  $\pi$ -stacking interactions ( $\sim 7$  Å<sup>25</sup>), disfavouring aromatic substrates. The transverse dimension and three-fold symmetry of the cavity are also consistent with a pyranose guest. Most importantly, the spacer units **3** are remarkably well-adapted for carbohydrate recognition. Each bis-urea unit adopts a twisted conformation due to H $\cdots$ H repulsion, and this positions them to form two H-bonds each to vicinal oxygen atoms (Fig 1c). When  $\beta$ -D-glucose **1** is introduced into the cavity this motif can form twice – once involving the 2- and 3-OH groups and secondly the 6-OH and pyranose ring O (Fig. 1d). Two more H-bonds can form between the third spacer and the glucose 4-OH, making ten in all. Of the polar groups in the complex, only one urea (in the third spacer) and the 1-OH are not involved in intermolecular H-bonding.



**Figure 1 | Design of glucose receptor 2.** **a**  $\beta$ -D-glucopyranose **1**, the predominant form of glucose in aqueous solution, highlighting the distinction between polar (red) and hydrophobic (blue) regions. **b** Formula of **2** employing the same colour coding, with water-solubilising groups in green. **c** The key H-bonding motif, involving diurea unit **3** and vicinal oxygen atoms. **d** The structure of **2.1** as predicted by Monte Carlo Molecular Mechanics (OPLS2005 force field). For details of the calculation see Supplementary Information. The complex features ten intermolecular hydrogen bonds, 1.95 - 2.48 Å in length, shown as yellow broken lines. The triethylmesitylene (TEM) units are coloured pale blue, and the dendrimeric side-chains are omitted for clarity.

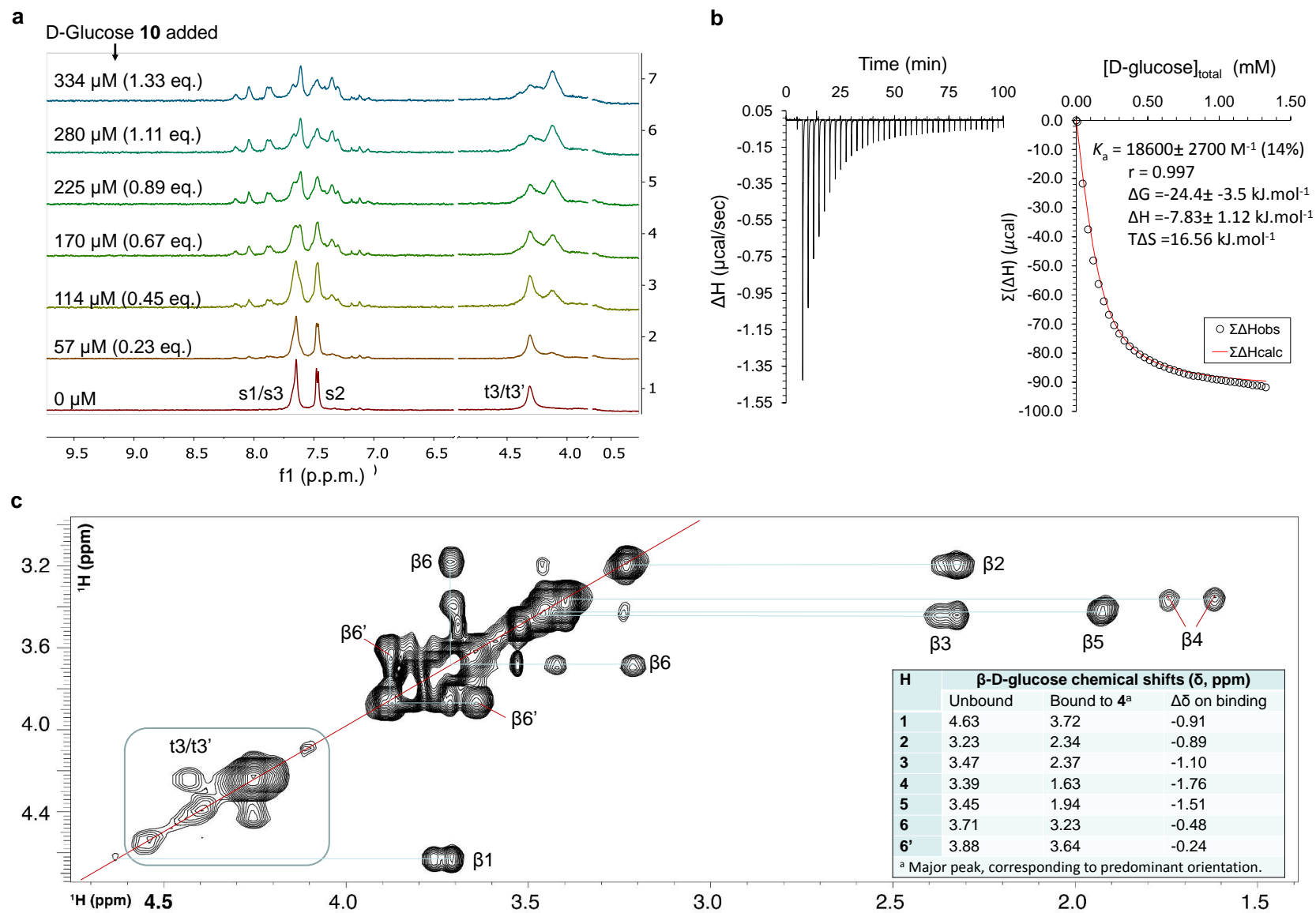
Bicyclic hexaurea **2** was prepared in 6 steps from known compounds **4**, **5** and **6**, as shown in Fig. 2. Notably, the key cyclisation of **7** + **8** gave low and unreliable yields in early experiments. Only when glycoside **9** was added as a template did the process become workable, occurring in ~50 % yield. The  $^1\text{H}$  NMR spectrum of **2** in  $\text{D}_2\text{O}$  reflected the symmetrical structure, with just three proton environments in the aromatic region (7.5-7.8 p.p.m.) (Fig. 3a). The spectrum was essentially unaltered between 0.05 and 1 mM, implying that the hexaurea is monomeric over this concentration range. NOESY spectra in  $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1 showed no cross-peaks between  $\text{NH}_{\text{B/B'}}$  and protons s1 or s3, consistent with the predicted “NH-in” conformation.





**Figure 2 | Synthetic route to receptor 2.** HBTU = 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, HOBT = 1-hydroxybenzotriazole, DIPEA = *N,N*-diisopropylethylamine, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, DMAP = 4-dimethylaminopyridine, TFA = trifluoroacetic acid. For details of procedures see Supplementary Information.

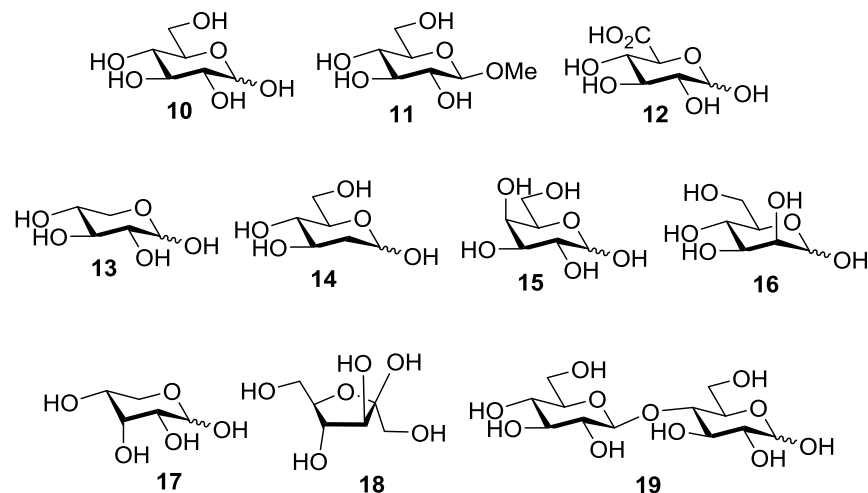
Addition of glucose to **2** in D<sub>2</sub>O caused major changes the NMR spectrum, as shown in Fig. 3a. A new set of signals appeared in the aromatic region, implying conversion of **2** to a less symmetrical structure. New peaks also appeared in the aliphatic region, especially around 4.2-4.5 p.p.m. The changes were consistent with complex formation which is slow on the <sup>1</sup>H NMR chemical shift timescale. Remarkably, they occurred well below mM concentrations of glucose, implying binding of unprecedented strength. Integration of the spectra allowed the affinity to be quantified as  $K_a = 18,000 \text{ M}^{-1}$ , nearly two orders of magnitude higher than observed for previous “synthetic lectins”. This affinity was confirmed by Isothermal Titration Calorimetry (ITC), which showed a strong exotherm analysed to give  $K_a = 18,600 \text{ M}^{-1}$  (Fig. 3b). NMR signals for bound glucose were obscured by receptor protons, but could be observed using two-dimensional methods. As shown in Fig. 3c, a ROESY spectrum showed chemical exchange peaks linking free and bound  $\beta$ -D-glucose **1**, revealing upfield movements of  $\sim 1.5$  p.p.m. on binding (see Table). Several protons exhibited two bound environments, consistent with the two orientations possible in the binding site. Upfield shifts for the glucose 6-CH<sub>2</sub> were relatively small, implying that the CH<sub>2</sub>OH protrudes from the cavity, as expected from modelling. The signals for the preferred bound state of **1** were also confirmed using a <sup>1</sup>H-<sup>13</sup>C HSQC spectrum. Neither spectrum contained peaks due to the bound  $\alpha$ -anomer of glucose, confirming the expected selectivity for all-equatorial substrates.



**Figure 3 | Evidence for binding of 2 to glucose 10.** T = 298 K throughout. For atom numbering, see Figure 1. **a** Partial  $^1\text{H}$  NMR spectra of **2** (0.25 mM) in  $\text{D}_2\text{O}$  (10 mM phosphate buffer, pH 7.4) with increasing quantities of D-glucose **10**. **b** ITC data and analysis curve for addition of glucose (7.5 mM) to **2** (0.13 mM) in water (buffered as for **a**). **c** Partial  $^1\text{H}$  NMR ROESY spectrum of receptor **2** (2 mM) with D-glucose (5 mM, 2.5 equivalents) in  $\text{D}_2\text{O}$ . Chemical exchange peaks (black, annotated) link CH protons on  $\beta$ -D-glucose **1** in free and bound states. Chemical shifts for the glucose protons, with signal movements due to binding, are listed in the table. Signals for bound  $\alpha$ -D-glucose were not observed under these conditions.

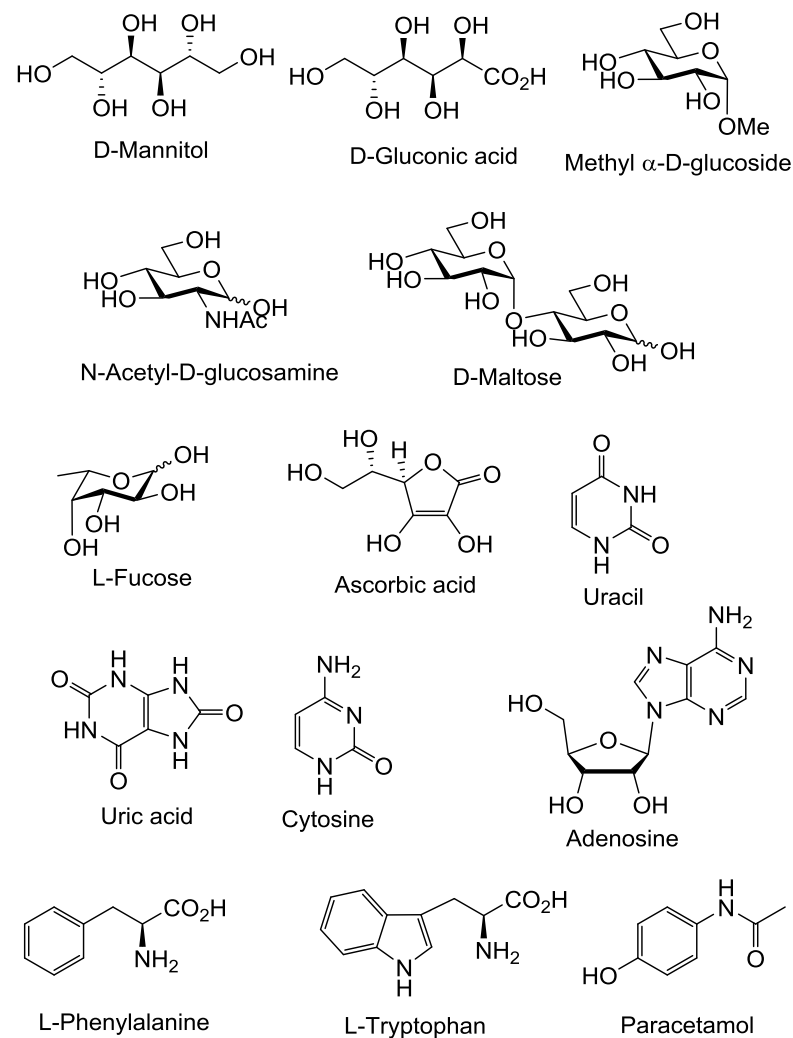
To assess the selectivity of receptor **2**, a variety of alternative substrates were tested using ITC and, where positive results were obtained, NMR titrations. The results are summarised in Fig. 4. Unsurprisingly, a few carbohydrates with close similarity to glucose **10** were also bound strongly. Methyl  $\beta$ -D-glucoside **11**, glucuronic acid **12** and xylose **13** possess pyranose structures with all-equatorial substitution patterns and show affinities  $<200\ \mu\text{M}$ . However, minor departures from the glucose structure can depress binding to a remarkable extent. Removing the 2-OH, as in 2-deoxyglucose **14**, reduces affinity by a factor of 25, even though the change introduces no steric effects. Inversion of a hydroxyl at positions 2 or 3, as in mannose **16** or galactose **15**, weakens binding by two orders of magnitude. Many of the substrates tested showed no evidence of binding by ITC (Fig. 4, right side). Based on the data for the weakest binders, it is likely that affinities down to  $\sim 20\ \text{M}^{-1}$  could have been detected (see Supplementary Information). On this basis **2** shows  $\geq 1000:1$  selectivity for glucose vs. “non-binding” substrates. The latter include carbohydrates such as the (all-equatorial) *N*-acetylglucosamine, as well as aromatic and heterocyclic compounds which might insert between the aromatic surfaces of **2**. Ascorbic acid and paracetamol, neither of which bind, are known to interfere with current glucose-sensing methodology<sup>26</sup>.

Binds to receptor 2



Substrate	$K_a$ ( $M^{-1}$ )	
	NMR	ITC
D-Glucose <b>10</b>	18,000	18,600
Methyl β-D-Glucoside <b>11</b>	7500	7900
D-Glucuronic Acid <b>12</b>	n.d. <sup>a</sup>	5300
D-Xylose <b>13</b>	n.d. <sup>a</sup>	5800
2-Deoxy-D-Glucose <b>14</b>	n.d. <sup>a</sup>	725
D-Galactose <b>15</b>	130	180
D-Mannose <b>16</b>	140	140
D-Ribose <b>17</b>	270	220
D-Fructose <b>18</b>	51	60
D-Cellobiose <b>19</b>	31	30

Binding minimal or undetectable



**Figure 4 | Substrates and affinities for receptor 2.** Affinities ( $K_a$ ) were measured in D<sub>2</sub>O (NMR) or H<sub>2</sub>O (ITC) containing phosphate buffer (10 mM, pH = 7.4) at T = 298 K. N.d. = not determined due to broadening of NMR signals on addition of substrate. For details of binding studies, see Supplementary Information.

If receptor **2** is to be used in biological contexts it must be able to tolerate mixtures of organic molecules, salts and variations in pH. ITC binding studies to glucose **10** were therefore conducted in a variety of media. In standard phosphate-buffered saline (PBS), titrations at pH = 6,7 and 8 gave  $K_a = 17,300\text{--}18,300\text{ M}^{-1}$ , essentially as for water. In cell culture media (DMEM, Leibovitz L-15) affinities were reduced to  $K_a \sim 5300\text{ M}^{-1}$ . However, both media contain substantial quantities of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , and control experiments indicated that these divalent cations were responsible for the weaker binding. Further work is required to establish how the cations diminish binding, but it seems unlikely that this factor will affect applications. To test **2** in human blood serum, it was first necessary to remove the large amount of endogenous glucose. This was achieved by oxidation with glucose oxidase + catalase, replacing the glucose with the non-binding gluconic acid (Fig. 4). After removal of high-MW components by dialysis, ITC gave  $K_a = 11,300\text{ M}^{-1}$  for **2+10** in this medium, only marginally lower than in water. Thermal stability and low toxicity are also important for applications. Receptor **2** showed no change by  $^1\text{H}$  NMR after heating to  $150\text{ }^\circ\text{C}$  for 1 hour, and no toxicity towards HeLa cells after 18 hours at up to 1 mM concentration.

It is instructive to compare the performance of receptor **2** with its counterparts from biology. In terms of affinity, the bacterial periplasmic glucose-binding proteins are significantly stronger (e.g.  $K_a \sim 5 \times 10^6\text{ M}^{-1}$  for the *E. coli* variant<sup>27</sup>). However, other classes of receptor proteins such as lectins<sup>14</sup> or glucose transporters<sup>28</sup> are comparable to **2** or weaker. Perhaps more importantly, the selectivity of **2** for glucose and closely-related



substrates is more typical of a biomolecule than a synthetic design. Relative to earlier synthetic systems, there is little doubt that the increased affinities result from the number and organisation of the H-bonding groups in the receptor. This work thus shows that, despite the challenges, hydrogen bonding can be rationally deployed to bind neutral polar molecules in aqueous solution. In practical terms, receptor **2** possesses a compact core which is easy to synthesise, stable, and seems to carry little risk of toxicity (unlike lectins such as Con A<sup>29</sup>). Its affinity should be sufficient for applications such as glucose monitoring<sup>9</sup> and glucose-responsive insulin<sup>10</sup> and, given its synthetic character, it should be readily adaptable for such purposes.

**Supplementary Information** is linked to the online version of the paper at [www.nature.com/nature](http://www.nature.com/nature).

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**Author Contributions** R.A.T. designed and carried out the synthetic route to receptor **2**. M.G.O. and J.V.M. assisted in optimisation of the synthesis of compound **7**. R.A.T. performed and analysed the binding studies, with assistance from T.S.C. and L.C. in some cases. R.A.T. and L.C. prepared the biological media. R.A.T. and M.P.C. were responsible for the structural NMR work, and H.L. performed the cytotoxicity studies. A.P.D. designed

the receptor and directed the study. The paper was written by A.P.D. with input from the other authors.

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